ChemComm

RSC Publishing

COMMUNICATION

View Article Online
View Journal | View Issue

Thiolated 2-methacryloyloxyethyl phosphorylcholine for an antifouling biosensor platform†

Cite this: *Chem. Commun.*, 2013, **49**, 8683

Received 10th June 2013,
Accepted 31st July 2013

DOI: 10.1039/c3cc44357d

www.rsc.org/chemcomm

Tatsuro Goda,*[a] Miyuki Tabata,[a] Mai Sanjoh,[a] Mai Uchimura,[b] Yasuhiko Iwasaki*[b] and Yuji Miyahara*[a]

We developed a new building block for a protein- and cell-repellant self-assembled monolayer (SAM) from 2-methacryloyloxyethyl phosphorylcholine (MPC) *via* a simple Michael-type addition to one mercapto group in alkanedithiol. The thiolated MPC can enable functionalization of a noble metal electrode to minimize noise signal in biosensing.

Reducing nonspecific interaction of biomolecules with an electrode is a key issue for sensitive and specific biosensing in biological matrices and for medical implants. In addition to polyethylene glycol (PEG), zwitterionic molecules containing a phosphobetaine, sulfobetaine, or carboxybetaine group have shown excellent biocompatibility by resisting undesired protein adsorption, cell attachment and biofilm formation in biomaterial applications.[1–4] Applying the biologically inert zwitterions to a terminal group of alkanethiol self-assembled monolayers (SAMs) results in formation of a robust thin film as an antifouling platform with a high degree of structural control on a metal electrode.[5–12] Such an antifouling interface allows us to focus on specific biomolecular recognition and biochemical reaction to an analyte.[13–16] Efforts have been made to synthesize zwitterionic alkanethiolates including phosphorylcholine (PC) alkanethiolate but the process is inefficient and time-consuming, requiring several steps under strictly anhydrous conditions and tedious purification processes.[6–9] Typically, the synthesis of PC alkanethiolate involves (1) protection of the mercapto group in hydroxyl-alkanethiol, (2) formation of cyclic phosphoesters at the hydroxyl group, (3) ring-opening reaction of cyclic phosphoesters by trimethylamine, and (4) deprotection of the mercapto group, with an overall yield of 63%.[6]

Here we report the one-step synthesis of phosphobetaine-type alkanethiolate from 2-methacryloyloxyethyl phosphorylcholine (MPC) as a building block for an antifouling SAM. MPC has been mainly used as a monomer to synthesize biomimetic polymer architectures by conventional or controlled radical polymerization techniques[17–19] whereas methacrylate also serves as an electron poor olefin (Michael acceptor). Thus, Michael-type addition efficiently occurs in between methacrylate and thiol groups under mild conditions.[20] We applied this thiol–ene reaction to functionalize one mercapto group in 1,6-hexanedithiol with MPC. The remaining mercapto group in MPC-SH can chemisorb to form a SAM on a gold surface (Fig. 1 and Fig. S1, ESI†). By optimizing the conditions, we successfully synthesized a thiolated MPC (MPC-SH).

ESI-TOF-MS analysis revealed that 88 mol% of the products were MPC-SH (m/z = 446.16) and its disulfide dimer (m/z = 891.32) (Fig. S2, ESI†). We obtained a small amount of the side product, 1,6-hexanedithiol with MPC at both ends (m/z = 741.27). But, this compound is not reactive to gold due to the absence of the thiol group. Only MPC-SH and its dimer formed a tightly packed SAM with a surface density of 3.7 ± 0.7 chains nm^{-2} (Fig. S3, ESI†). This value is slightly lower than that of conventional alkanethiol SAMs[21] because of the geometric constraint by the bulky PC headgroup. Self-assembling aligns MPC-SH molecules similarly to the phospholipid monolayer, orienting the zwitterionic PC moiety to the water phase. Formation of MPC-SH SAM tolerated the surface impedance that does not passivate the underlying electrode in electrochemical sensing (Fig. S4 and S5, Table S1, ESI†).

To confirm antifouling properties of the SAM, we measured the amount of nonspecific adsorption of protein using a quartz crystal microbalance (QCM) and semiconductor-based potentiometric sensors in a real-time and label-free manner. The potentiometric sensor gives us changes in the interface potential (ΔV) caused by adsorption of innately charged protein within an electrical double layer capacitor (C, Table S1, ESI†).[22,23] The adsorbed mass of protein (Γ) was calculated from the QCM results using Saueabrey's equation.[24] The adsorbed amounts of bovine serum albumin (BSA), bovine fibrinogen,

[a] *Institute of Biomaterial and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda, Tokyo 101-0062, Japan. E-mail: goda.bsr@tmd.ac.jp, miyahara.bsr@tmd.ac.jp*

[b] *Faculty of Chemistry, Materials and Bioengineering, Kansai University, 3-3-35 Yamate-cho, Suita, Osaka 564-8680, Japan. E-mail: yasu.bmt@kansai-u.ac.jp*

† Electronic supplementary information (ESI) available: Synthesis and characterization of MPC-SH, SAM formation and characterization; and protein and cell measurements. See DOI: 10.1039/c3cc44357d

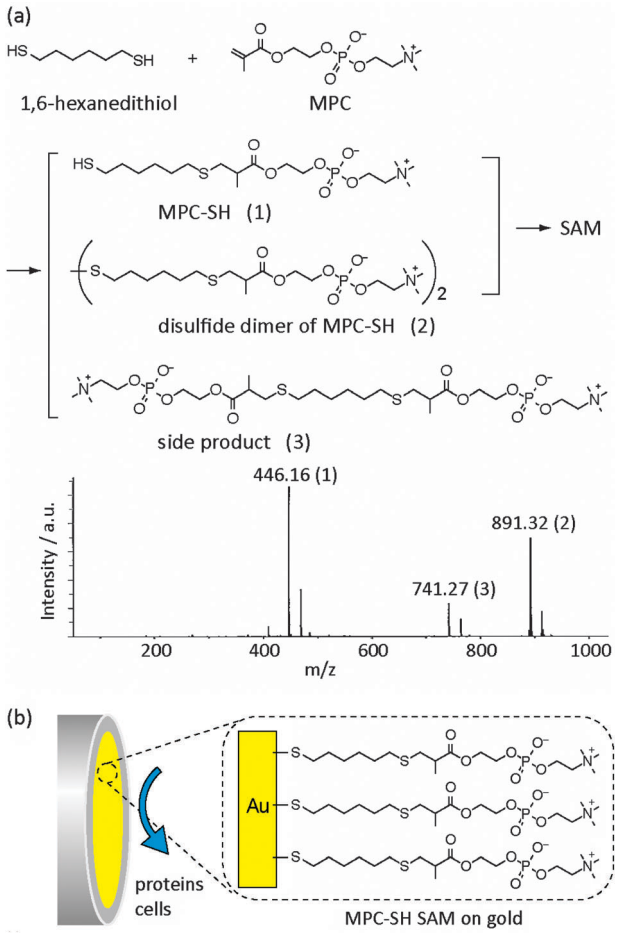

Fig. 1 (a) Synthetic route of MPC-SH and its characterization by ESI-TOF-MS. (b) Schematic representation of antifouling MPC-SH SAM on gold electrode.

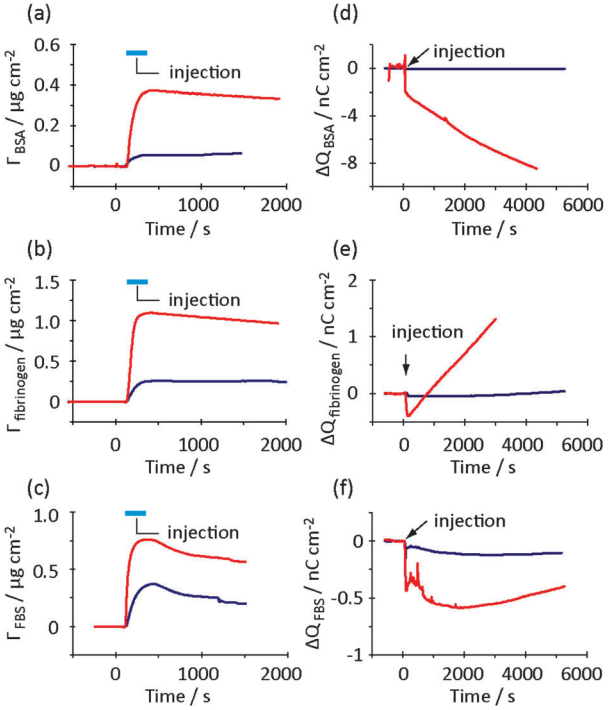

Fig. 2 Real-time and label-free quantification of nonspecific protein adsorption on the MPC-SH SAM-coated (blue) and unmodified (red) gold electrodes using QCM (a–c) and potentiometric (d–f) biosensors: in 1 mg mL^{-1} BSA (a and d), 0.3 mg mL^{-1} fibrinogen (b and e), and 10% FBS (c and f).

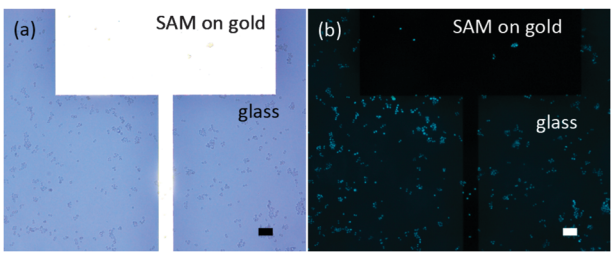

Fig. 3 Adhesion of HepG2 cells on micro-patterned gold electrodes with MPC-SH SAM on a glass substrate; bright field (a) and nuclei-stained (b) images at the same position. Scale bar: 100 μm.

and fetal bovine serum (FBS) components were significantly reduced on the surface of the MPC-SH SAM (Fig. 2). Trends were similar in the charge sensing of proteins by the potentiometry ($\Delta Q = C\Delta V$). The reduced electric signals are attributed to the lowered amount of bound proteins on the tethered gold electrode. The directions of ΔQ were dependent on local positive or negative net-charges of the adsorbed proteins within the electrical double layer in the buffer solution (1× Dulbecco's PBS, pH 7.4). The net-charges of proteins located outside of the double layer cannot be detected because of the charge-screening effect by mobile counterions.

Finally, we seeded model cells on micro-patterned gold electrodes on a glass substrate. The cells attached solely to the glass substrate, whereas almost no cells adhered to the electrode coated with the MPC-SH SAM (Fig. 3 and Fig. S6, ESI†). We consider that the protein repellency prevent cell adhesion because cells require adsorbed proteins as a scaffold to attach.

The reason for biologically inert properties of a MPC-SH SAM is the formation of a strong hydration layer above the densely packed monolayer that creates a repulsive force to protein molecules at the interface.[9,25] Water molecules are tightly retained around PC with bulk water-like mobility. Exhibiting surface charge neutrality of zwitterionic PC also diminishes electrostatic interaction with proteins. With a sharp contrast to sulfobetaine, antifouling characteristic by PC is effective under a wide range of pH and ionic strength. Sulfobetaine requires a high ionic strength (>200 mM) and mild alkaline conditions for exhibiting protein repellency.[5,26] Compared with conventional antifouling PEG, the zwitterionic molecules are less prone to auto-oxidation in most biochemically relevant solutions.[27,28]

Reducing nonspecific interaction is in particular essential to label-free technologies that are advantageous due to their simplicity over conventional labeling methods. A target species should be selectively captured because label-free sensors detect any molecules placed on a sensing area. A mixed SAM, composed of target-capturing element and backfilling alkanethiol, is frequently used as a designed functional thin film.[13] Applying MPC-SH as a backfilling agent to reduce nonspecific interaction

would enable a sensitive and specific detection in a realistic dirty sample.

In conclusion, we present a simple synthesis of MPC-SH as a new element for preparing antifouling SAMs against proteins and cells. The biologically inert platform meets the requirements for successful biosensing and clinical implants.

This research is supported in part by the JST-CREST, FIRST program, JSPS Grant-in-Aid for Young Researcher B (23750079), and Grant-in-Aid on Scientific Research for Innovative Areas (2306).

Notes and references

1 Y. Iwasaki and K. Ishihara, *Anal. Bioanal. Chem.*, 2005, **381**, 534–546.
2 R. S. Smith, Z. Zhang, M. Bouchard, J. Li, H. S. Lapp, G. R. Brotske, D. L. Lucchino, D. Weaver, L. A. Roth, A. Coury, J. Biggerstaff, S. Sukavaneshvar, R. Langer and C. Loose, *Sci. Transl. Med.*, 2012, **4**, 153ra132.
3 H. Chen, L. Yuan, W. Song, Z. K. Wu and D. Li, *Prog. Polym. Sci.*, 2008, **33**, 1059–1087.
4 Z. Zhang, M. Zhang, S. Chen, T. A. Horbett, B. D. Ratner and S. Jiang, *Biomaterials*, 2008, **29**, 4285–4291.
5 R. E. Holmlin, X. X. Chen, R. G. Chapman, S. Takayama and G. M. Whitesides, *Langmuir*, 2001, **17**, 2841–2850.
6 V. A. Tegoulia, W. S. Rao, A. T. Kalambur, J. R. Rabolt and S. L. Cooper, *Langmuir*, 2001, **17**, 4396–4404.
7 Y. C. Chung, Y. H. Chiu, Y. W. Wu and Y. T. Tao, *Biomaterials*, 2005, **26**, 2313–2324.
8 S. F. Chen, L. Y. Liu and S. Y. Jiang, *Langmuir*, 2006, **22**, 2418–2421.
9 S. F. Chen, J. Zheng, L. Y. Li and S. Y. Jiang, *J. Am. Chem. Soc.*, 2005, **127**, 14473–14478.
10 S. F. Chen and S. Y. Jiang, *Adv. Mater.*, 2008, **20**, 335–338.
11 Q. Jin, J. P. Xu, J. Ji and J. C. Shen, *Chem. Commun.*, 2008, 3058–3060.
12 W. B. Zhou, J. Y. Shao, J. Qiao, Q. S. Wei, J. G. Tang and J. Jian, *Chem. Commun.*, 2010, **46**, 1479–1481.
13 T. Goda, K. Masuno, J. Nishida, N. Kosaka, T. Ochiya, A. Matsumoto and Y. Miyahara, *Chem. Commun.*, 2012, **48**, 11942–11944.
14 T. Goda and Y. Miyahara, *Biosens. Bioelectron.*, 2013, **45**, 89–94.
15 H. Vaisocherova, W. Yang, Z. Zhang, Z. Cao, G. Cheng, M. Piliarik, J. Homola and S. Jiang, *Anal. Chem.*, 2008, **80**, 7894–7901.
16 A. L. Gui, E. Luais, J. R. Peterson and J. J. Gooding, *ACS Appl. Mater. Interfaces*, 2013, **5**, 4827–4835.
17 Y. Iwasaki and K. Ishihara, *Sci. Technol. Adv. Mater.*, 2012, **13**, 064101.
18 R. Matsuno and K. Ishihara, *Nano Today*, 2011, **6**, 61–74.
19 R. Iwata, P. Suk-In, V. P. Hoven, A. Takahara, K. Akiyoshi and Y. Iwasaki, *Biomacromolecules*, 2004, **5**, 2308–2314.
20 R. Matsuno, K. Takami and K. Ishihara, *Langmuir*, 2010, **26**, 13028–13032.
21 G. E. Poirier and M. J. Tarlov, *Langmuir*, 1994, **10**, 2853–2856.
22 T. Goda and Y. Miyahara, *Langmuir*, 2012, **28**, 14730–14738.
23 T. Goda and Y. Miyahara, *Anal. Chem.*, 2010, **82**, 1803–1810.
24 M. Muratsugu, F. Ohta, Y. Miya, T. Hosokawa, S. Kurosawa, N. Kamo and H. Ikeda, *Anal. Chem.*, 1993, **65**, 2933–2937.
25 Y. He, J. Hower, S. F. Chen, M. T. Bernards, Y. Chang and S. Y. Jiang, *Langmuir*, 2008, **24**, 10358–10364.
26 E. Ostuni, R. G. Chapman, M. N. Liang, G. Meluleni, G. Pier, D. E. Ingber and G. M. Whitesides, *Langmuir*, 2001, **17**, 6336–6343.
27 Y. Y. Luk, M. Kato and M. Mrksich, *Langmuir*, 2000, **16**, 9604–9608.
28 T. A. Snyder, H. Tsukui, S. Kihara, T. Akimoto, K. N. Litwak, M. V. Kameneva, K. Yamazaki and W. R. Wagner, *J. Biomed. Mater. Res., Part A*, 2007, **81**, 85–92.